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EXAMINER	
BULL, CHRISTOPHER	
ART UNIT	PAPER NUMBER
1655	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/501,131	BLACKMAN ET AL.	
	Examiner	Art Unit	
	Christopher Bull	1655	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *30 December 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-33* is/are pending in the application.

4a) Of the above claim(s) *21-23 and 29-32* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-20,24-28 and 33* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *1/05&1/06*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

The abbreviation TMR will be used for tetramethylrhodamine.

Cl# will be used to refer to a claim number in a reference, to avoid confusion with Claims in the instant application.

Election/Restrictions

Applicant's election with traverse of Group I in the reply filed on 30 Dec 2005 is acknowledged. The traversal is on the ground(s) that unity of invention practice was improperly followed because inventions III and IV were not kept with invention I (page 3). A thorough consideration of applicants arguments and the disclosure has altered the basis for the lack of unity restriction from *a priori* to *a posteriori,* due to an unrecognized ambiguity in the language of Claim 1, but the result is identical.

The language of Claim 1 is subject to two alternative interpretations. If it is read as drawn to a peptide containing two cysteine residues, each labeled via its thiol with its own alkyleneamidoTMR group (i.e., two TMRs per peptide), then the application would have unity *a priori*, with Claim 1 providing the special technical feature. The Examiner initially read Claim 1 as drawn to a peptide containing two cysteine residues, each labeled via its thiol, to "an" (meaning one) alkyleneamidoTMR group, (i.e., one shared TMR per peptide, linked through both thiols). The application then lacks unity *a priori*, when both the latter compound (as Claim 1) and the former (as Claim 21) are claimed. It appears that applicants intended only the former reading of Claim 1 (i.e., two TMRs per peptide, each singly attached to Cys).

Assuming two TMRs per peptide in Claim 1, the application lacks unity because the genus of substrates recited by Claim 1 lacks novelty and inventive step over Komoriya & Packard (US 6,037,137, issued 14 Mar 2000). This reference claims a genus of just such fluorogenic protease substrates (see Cl#s 1, 5, 7 and 8), based on reacting an iodoacetamideTMR derivative (see Col 25 lines 9-13) with two cysteine residues (see Formula VII in Col 21) on opposite sides of a proteolytic cleavage site (Col 15, lines 40-67). These substrates function by taking advantage of substantial fluorescence quenching due to formation of ground state dimers in the uncleaved substrate that disappear upon proteolysis (Col 22-23). There remains no novel common technical feature to provide unity of invention between Claim 1 (a doubly labeled fluorogenic substrate, labeled via thiols but requiring no substantial isomeric identity in labels or linkages) and independent claims: 21 (a doubly labeled fluorogenic substrate, differing from Claim 1 in allowing any type of linkage but requiring substantial isomeric identity in labels and linkages); 29 (kit); and 31 (solid support).

Applicants also traverse the restriction requirement based on two further arguments. First (pages 4-5), that it should not matter whether claims drawn to the same invention are expressed as independent claims or depend from a single independent claim, if that claim were found to be novel and inventive over the prior art. Since that is not the case, that argument is now moot. The second (pages 5-6) is that examining all together would be little burden. Since establishing burden is strictly a US practice, and has no bearing on deciding on unity of invention, this is not persuasive. It should be noted that there is indeed a burden due to examining more than one

invention not linked by a special technical feature. Thus, the arguments against restriction are not found persuasive.

As in the original restriction requirement, the first product (Claim 1) and the first recited method of making that product (Claim 18) and of using that product (Claim 24) constitute the first invention. Inventions II, III and IV remain the same as well. Applicants have elected Invention I. To recapitulate, the Inventions are:

I [bis(thioalkyleneamidoTMR)peptide + making + using] Claims 1-20, 24-28 & 33;

II [bis(TMR)peptide with isomeric identity of label & linkage] Claims 21-23;

III [Kit with standard protease composition] Claims 29-30;

IV [Solid support for immobilized substrate] Claims 31-32.

The requirement is still deemed proper and is therefore made FINAL.

Claims 1-20, 24-28 and 33 are presented for examination on the Merits.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-20, 24-8 and 33 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 is subject to two alternative interpretations as discussed above: the peptide of Claim 1 may contain two cysteine residues, each labeled via its thiol with its

own alkyleneamidoTMR group (i.e., two TMRs per peptide), or may contain two cysteine residues, each labeled via its thiol, to "an" (meaning one) alkyleneamidoTMR group, (i.e., one shared TMR per peptide, linked through both thiols). The specification (page 3, lines 17-23) suggests that the former interpretation (i.e., two TMRs per peptide) is intended, but nowhere states that it is required. Chemical intuition and logic also favor the former, but do not preclude the latter. Accordingly, Claim 1 as instantly drafted, and claims that refer to or depend from Claim 1, fail to distinctly claim the subject matter.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-3, 8-12, 14-17, 24-25, 27 and 33 are rejected under 35 U.S.C. 102(b) as being anticipated by Komoriya et al. (US 6,037,137 issued 14 Mar 2000).

Komoriya et al. teach fluorogenic protease substrates (see Cl#s 1, 5, 7 and 8) with various protease recognition sites (Tables 2 and 4, Col 13-20), including a genus with two cysteine residues (see Formula VII in Col 21) on opposite sides of a proteolytic cleavage site (Col 15, lines 40-67) that is made by reacting the thiols with an iodoacetamideTMR derivative (see Col 25 lines 9-13). They also teach that these substrates function by taking advantage of substantial fluorescence quenching due to formation of ground state dimers in the uncleaved substrate, that disappear upon proteolysis (Col 22-23).

Claim 1 is drawn to a genus of fluorogenic protease substrates comprising peptides having two thiol groups, wherein each said thiol group is labeled with an alkyleneamidoTMR. In Komoriya et al., the fluorophores, F1 and F2, are attached at aa1 and aa10, wherein "aa1 and aa10 are independently selected from the group consisting of lysine, ornithine and cysteine" (Cl# 1, Col 215 lines 56-57). Although a genus need not anticipate a species when the genus is quite broad with respect to the species, that is not the case here: fully 1/6 of the genus of the reference reads on the instant claim. Komoriya et al. are quite explicit in teaching that F1 and F2 may each be attached through the thiol of a cysteine (Cl#s 7 and 8) using an iodoacetamidoTMR (Formula VII in Col 21) and provide working examples of F2 so attached (Examples 1 and 3, Cols 33 and 39). Turning to succeeding Claims in the instant application:

Claim 2 recites that the alkyleneamidoTMR groups be the same. Cl# 5 of the reference recites that F1 and F2 be the same fluorophore.

Claim 3 recites that the alkyleneamidoTMR group be methyleneamidoTMR, as shown by the reference in Formula VII (Col 21).

Claims 8-12 and 14-17 recite various limitation as to protease recognition site length, location between the fluorophores, and content of cysteine residues. Cl# 1 of the reference reads on each of these claims.

Claims 24-5, 27 and 33 are drawn to methods of assaying protease activity by contacting samples, including intact cells and tissue to the substrate. Cl#s 35-37 of the reference read directly on these claims (Col 219-220).

Accordingly, the cited reference is deemed to anticipate the inventions of Claims 1-3, 8-12, 14-17, 24-25, 27-28 and 33.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-3, 8-12, 14-20, 24-28 and 33 are rejected under 35 U.S.C. 103(a) as being unpatentable over Komoriya et al.

Claims 18-20 recite methods of making the fluorogenic peptide substrate. Claim 26 recites a method pH limitation between about 5 and 10, while Claim 28 requires assaying a protease with a known recognition site using a fluorogenic peptide.

The teachings of Komoriya et al. have been discussed above and are applied as before with respect to Claims 1-3, 8-12, 14-17, 24-25, 27 and 33. Komoriya et al. also beneficially teach that it is better to use tetramethylrhodamine labels than X-rhodamine labels because the former give a larger fluorescent enhancement upon cleavage (Example 18, Col 50 L 62 – Col 51 L 14). They further beneficially recommend assays at pH 8.9 (Example 12 Col 47), at pH 7.5 (Example 3 Col 39), and at a protease pH optimum (Col 27 lines 1-6). Komoriya et al. further beneficially teach an assay for elastase (Example 3, Col 39) using a fluorogenic peptide containing one acetamidoTMR labeled cysteine.

Komoriya et al. do not show reacting an unlabelled peptide containing two cysteine groups with iodoacetamideTMR, nor reciting a pH range limitation, nor give an example of using a fluorogenic substrate with widely spaced identical cysteine labels to assay a known protease.

It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to employ iodoacetamideTMR to label the unlabelled peptides of Komoriya et al. that had widely spaced cysteine residues via the instantly claimed steps, because it is within the ordinary skill in the art to prefer a single step synthesis over a multistep process (particularly react, deblock, and react again with same reagent), and Komoriya et al. teach that it is within the ordinary skill in the art to

make a fluorogenic protease substrate by labeling cysteines on opposite sides of a protease recognition site, and also that labeling with TMR is preferable. This combination reads on the matter of Claims 18-20.

It would also have been obvious to one of ordinary skill in the art at the time the claimed invention was made to employ fluorogenic substrates with widely spaced cysteine labels in the methods of Komoriya et al. that use pH values of 8.9 and 7.5 via the instantly claimed steps, because Komoriya et al. teach that it is within the ordinary skill in the art to such substrates at those pH values. This combination reads on the matter of Claims 26.

It would also have been obvious to one of ordinary skill in the art at the time the claimed invention was made to employ fluorogenic substrates with widely spaced cysteine labels on either side of known protease recognition sites in the methods of Komoriya et al. via the instantly claimed steps, because Komoriya et al. teach that it is within the ordinary skill in the art to use such substrates to assay for specific proteases. This combination reads on the matter of Claims 28.

One would have been motivated to do so for the expected benefit of a simpler synthesis, and because Komoriya et al. show facile labeling with iodoacetamideTMR derivatives. Komoriya et al. also presented pH values of 7.5 and 8.9 and known protease recognition sites as beneficial to the assay.

Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 1-12, 14-20, 24-28 and 33 are rejected under 35 U.S.C. 103(a) as being unpatentable over Komoriya et al. in view of Packard et al. (J Phys. Chem. B 102, 1820-1827 pub 14 Feb 1998).

Claims 4-7 recite added limitations as to isomeric purity of TMR derivative.

The teachings of Komoriya et al. have been discussed above and are applied as before with respect to Claims 1-3, 8-12, 14-20, 24-28 and 33.

Komoriya et al. do not teach using substantially isomeric identity in label.

Packard et al. studied fluorogenic protease substrates labeled at each end with the same TMR group. They teach that increased length in the peptide loop gives greater fluorescent enhancement upon cleavage (Table 2, page 1824). They beneficially teach (in the same length peptide) that using isomerically pure 6-labelled TMR, as opposed to isomerically pure 5-labelled TMR, gives a greater fluorescent enhancement upon cleavage (Table 3, page 1825), nearly nine-fold.

It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to employ a substantially isomerically pure TMR reagent in making and using the fluorogenic peptides of Komoriya et al., via the instantly claimed steps because Packard et al. teach that it is within the ordinary skill in the art to prefer a 6-substituted TMR reagent to a mixture of 5- and 6-isomers or the 5-isomer, and Komoriya et al. teach that it is within the ordinary skill in the art to use a fluorogenic protease substrate containing labels at two cysteines on opposite sides of a protease

recognition site to conduct assays for proteolytic enzymes. This combination reads on the matter of Claims 4-7.

One would have been motivated to do so for the expected benefit of an assay with increased sensitivity to cleavage due to stronger quenching before cleavage (less background signal).

Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 1-20, 24-28 and 33 are rejected under 35 U.S.C. 103(a) as being unpatentable over Komoriya et al. in view of Packard et al, as applied to claims 1-12, 14-20, 24-28 and 33 above, and further in view of Wei et al. (US 6,787,329 issued 7 Sept 2004 but filed 24 Nov 1999).

Claim 13 recites an added limitation as to lack of well-defined conformation, determinable by NMR.

The teachings of Komoriya et al. in view of Packard et al. have been discussed above and are applied as before with respect to Claims 1-12, 14-20, 24-28 and 33. Komoriya et al. beneficially show that increasing flexibility leads to faster cleavage by the protease (Col 49-50). Packard et al. beneficially show that longer peptides are better than shorter (Table 2, page 1824).

Both references are silent as to NMR characterization of the backbone.

Wei et al. teach fluorogenic protease substrates containing self-quenching TMR groups on either side of a protease recognition site (Col 4-5). Wei et al. teach that flexibility in the peptide backbone is greatly to be desired, contrary to previous teachings of the Packard group (Cols 6-7). Proteolysis of their bis(aminoTMR)-labeled substrate induced a 28-fold increase in fluorescence (Table I, col 11-12), very comparable to the 25-fold increase reported by Applicants (page 3 lines 5-15). Given the similarity in performance, the flexibility recommended by Wei et al. in the peptide backbone is taken as equivalent to the lack of well-defined conformation recited in Claim 18, especially in the absence of evidence to the contrary. Applicant's report (Disclosure page 18) of certain NMR parameters cannot be taken as a limitation, absent any comparative data. .

It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to employ a flexible backbone as taught by Wei et al. while making and using bis(thioalkyleneamidoTMR) labeled fluorogenic peptides as taught by the combination of Komoriya et al. and Packard et al. via the instantly claimed steps, because Wei et al. teach that it is within the ordinary skill in the art to incorporate flexibility in backbone, and the combination of Komoriya et al. in view of Packard et al. shows that it is within the ordinary skill in the art to use a fluorogenic protease substrate containing labels at two cysteines on opposite sides of a protease recognition site to conduct assays for proteolytic enzymes. This combination reads on Claim 13.

One would have been motivated to do so for the expected benefit of an assay with increased sensitivity due better quenching in the uncleaved substrate (i.e., lower background fluorescence).

Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Conclusion

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Burroughs-Tencza (US 2003/0166028 published 4 Sept 2004 but filed 9 Feb 2001) shows doubly labeled protease peptides containing one cysteine labeled rhodamine derivative as an acceptor, using iodoacetamide linkers.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Christopher Bull whose telephone number is (571) 272-1327. The examiner can normally be reached on 7:30-4.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Terry McKelvey can be reached on (571) 272-0775. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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